IQNS-monitored dynamical transition of a small β-protein following heat denaturation

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Abstract

Quasi-elastic neutron scattering experiments performed on neocarzinostatin, a small β-protein, reveal a drastic change in the fast (picosecond time scale) diffusive internal dynamics when the protein unfolds by heating. Data treatment is based on a model that separates contributions arising from global and internal motions. Most of internal dynamic parameters (amplitude of diffusive motions, fraction of "immobile" scatters, mean-square vibrational amplitude) undergo abrupt modifications when the temperature is raised above 65°C. © 2000 Elsevier Science B.V. All rights reserved.

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We present here the dynamic changes that arise during the first steps of heat denaturation of neocarzinostatin (NCS), a small β-protein composed of 113 residues stabilised by two disulphide bridges [1], using incoherent quasi-elastic neutron scattering. Previous studies show that the globular protein (radius of gyration $R_g = 14$ Å) begins to unfold at 66°C in D_2O buffer and is completely unfolded at 78°C, with a Kratky–Porod chain behaviour ($R_g = 26.3$ Å). The transition midpoint is $T_m = 71°C$ [2].

The present measurements were performed between 20°C and 71°C on the Mibémol time-of-flight spectrometer of the Orphée reactor, Saclay, France. The spectrometer operated with an incident energy of 2.27 meV, a wavevector range of $0.3 < Q < 2.0$ Å^{-1} and an energy resolution of 0.0098 meV (FWHM). The sample concentration was about 50 mg/ml in a 60 mM phosphate deuterated buffer. The analysis was made using a model developed for a native protein which decouples the local motions (arising from conformational changes of the side chains) and the Brownian motions of the whole protein in the solution [3]. We have shown by single value

decomposition of small-angle X-ray scattering data that at 71°C the solution contains about 50% of protein in a native-like state and 50% in an intermediate state which is not completely unfolded ($R_g = 24$ Å) since its scattering profile indicates the presence of some structured parts. Thus, we assume that the model used for native proteins is still approximately valid until 71°C. The dynamical incoherent structure factor is described from

$$S(Q, \omega) = e^{-\langle u^2 \rangle Q^2/3}[L_1(Q, \omega) \otimes \{A_0(Q)\delta(\omega)$$
$$+ (1 - A_0(Q))L_2(Q)\}].$$

The Lorentzian L_1, of width $\Gamma(Q)$, describes the Brownian motions of the whole protein in solution. The Lorentzian L_2 describes the internal confined diffusive and reorientational motions. $\langle u^2 \rangle$ is the vibrational mean-square displacement.

From the analysis of the first Lorentzian we have been able to extrapolate the diffusion coefficient (D_s, given by $\Gamma(Q) = D_s Q^2$) of the protein as a function of temperature (Fig. 1). In the case of a globular protein, D_s is approximately related to the Einstein diffusion coefficient D_{tr} ($D_{tr} = kT/6\pi\eta R_H$, where η is the buffer viscosity and $R_H = 1.29 R_g$ the hydrodynamic radius) by the relation $D_s = 1.27 D_{tr}$ [3]. As shown in Fig. 1 the values obtained

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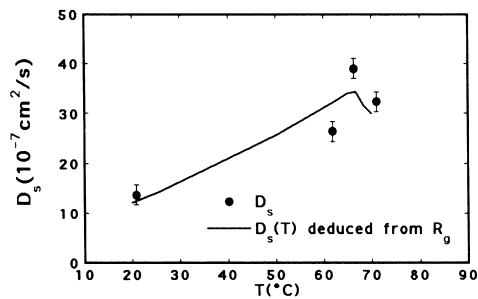*E-mail address:* daniela@bali.saclay.cea.fr (D. Russo)

Fig. 1. Experimental values of D_s as a function of T (circles) and $D_s(T)$ curve obtained from $R_g(T)$ data (solid line).

Table 1
Evolution with T of p, δ and $\langle u^2 \rangle$

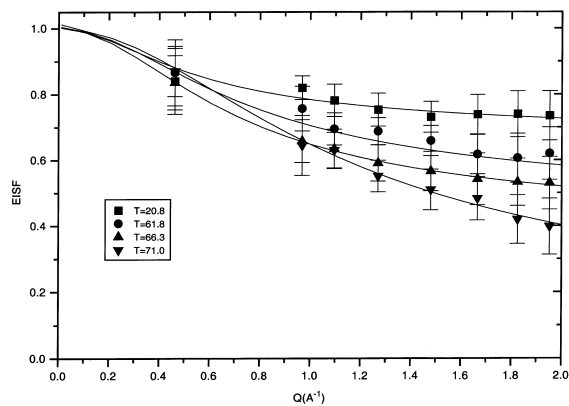| T (°C)	20.8	61.8	66.3	71.0
p	0.67 ± 0.02	0.45 ± 0.02	0.39 ± 0.03	0.09 ± 0.03
δ (Å)	3.14 ± 0.10	2.23 ± 0.05	2.12 ± 0.05	1.48 ± 0.07
$\langle u^2 \rangle$ (Å^2)	0.12 ± 0.01	0.15 ± 0.02	0.21 ± 0.03	0.22 ± 0.03

Fig. 2. Values of A_0 as a function of Q and fitting lines resulting from the model of free diffusion in a sphere.

for D_s are in good agreement with the $D_s(T)$ curve deduced via the previous relations from our measurements of R_g by SAXS.

Significant evolutions with temperature are also detected in the protein internal dynamics. To characterise the evolution of A_0 (Fig. 2), which depends on the volume explored by the atoms, we used the model of free diffusion in a sphere of finite size [4], with a distribution of sphere diameters. Two parameters are determined: δ and p, where p represents the fraction of hydrogens which do not undergo any detectable diffusive motion in

the Q, ω accessible domain [5] and δ gives the average value of the radius of the sphere in which diffusion occurs (see Table 1).

Both values of p and δ decrease by about 30% from 20°C to 62°C, where the protein remains globular. At room temperature, the dominant contribution to the internal motions primarily arise from the exposed side chains, while with increased temperature the buried hydrogens, which are for the most part in β-sheet structures, also contribute, which explains why p diminishes. The fact that these latter hydrogens move in a more constrained environment also explains the decreasing of the δ value. The drastic diminution of p at the transition could be interpreted by the hypothesis that almost all hydrogens become mobile as the protein unfolds.

The value of the width Γ_2 of the "internal" does not seem to be very dependent on Q and temperature. But the determination of Γ^2 is inaccurate and no conclusion can be deduced from this behaviour.

The vibrational mean square displacement $\langle u^2 \rangle$ almost follows a harmonic law until 62°C, then abruptly increases. This can be the result of a changing in the potential of the protein, when all hydrogen bonds are disrupted.

References

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